al 1986) and as such the activity may appear to be independent of cutaneous viability. Metabolism by the cutaneous microflora may also appear to be independent of viability. However, as with benzo[a]pyrene, the apparent extent of absorption of glycerol trioleate will be dependent on the extent of metabolism.

The data from this experiment imply that the use of a standard set of conditions developed for measuring the in-vitro percutaneous absorption of one compound cannot necessarily be used for other compounds with different physicochemical properties. Use of inappropriate conditions will lead to erroneous assumptions as to the ability of chemicals to permeate the skin.

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Action of a chronic arecoline administration on mouse motility and on acetylcholine concentrations in the CNS

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Abstract—The modifications of mouse motility and of the levels of acetylcholine (ACh) in two sections of the CNS caused by a chronic administration of 4·5; 9·5; 28·5 and 60 mg kg^{-1} day^{-1} of arecoline for 20 days have been studied. At low doses (4·5 and 9·5 mg kg^{-1} day^{-1}), arecoline caused no modification of the ACh levels and of the motility. The higher doses (28·5 and 60 mg kg^{-1} day^{-1}) caused a reduction of the mouse motility and an increase of the ACh levels in the subcortical structures of the CNS of the mouse.

There is evidence that the administration of arecoline may improve memory (Bartus et al 1980; Flood et al 1984). Pharmacological alleviation of memory disorders necessarily involves the prolonged use of drugs and it is important to verify the incidence of collateral effects in a chronic drug administration. With these premises we studied in the mouse the modifications of motor activity and of the levels of acetylcholine (ACh) in the CNS after a chronic arecoline administration.

Methods

Female albino "Swiss Morini" mice, 20–25 g, were housed in groups of 20. Because of the short duration of the arecoline effect (Pradham & Dutta 1970), frequent drug administration was needed, therefore, arecoline was dissolved in the drinking water. The concentrations used were: 0·05, 0·1, 0·4 and 0·7 gL^{-1}. The four experimental groups (of 20 mice each) drank the various arecoline solutions and the fifth group drank water (controls).

The pharmacological treatment lasted 20 days. In this period, the mean liquid consumption for each mouse was 2·02 mL^{-1} in the controls and 2·00 mL day^{-1} at 0·05 gL^{-1}, 2·10 mL day^{-1} at 0·1 gL^{-1}, 1·78 mL day^{-1} at 0·4 gL^{-1} and 1·95 mL day^{-1} at 0·7 gL^{-1} of arecoline.

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A modification of the open field for rats (Brimblecombe 1963) was used to evaluate the spontaneous mouse activity. The floor of a square box (side 35 cm, peripheral wall 25 cm high) was divided in 25 squares (side 7 cm). The floor was covered with a transparent plastic sheet to facilitate its cleaning. A lamp (80 W) at a distance of 150 cm gave a uniform illumination of the box floor. The mice were tested individually. To reduce or to eliminate the initial exploratory activity which may interfere with the evaluation of the simple motor activity, the mouse was put in the centre of the box for 10 min; in the subsequent 5 min period the number of squares crossed was determined. A square was considered crossed only when the animal entered the square with all four paws. All experiments were performed in the morning at the end of the chronic pharmacological treatment. The mice still had the drug concentrations at the time of testing.

The same day in which the test of motility was performed, at least five mice of the various experimental groups were killed by microwave irradiation of the head. The skull was opened and the brain frozen (−30°C). The brain was cut through the crus cerebri; cerebellum and pons were discarded. The cortex was collected and weighed. The remaining part of the brain (subcortex) was also weighed. ACh was extracted by the method given by Beani and Bianchi (1964). The tissue after homogenization in 2 mL of McIlvaine's citric disodium phosphate buffer (0·014 M; pH 4), was kept for 30 s in boiling water, then transferred to ice cold water and diluted with an equal volume of frog Ringer solution containing eserine salycilate (2×10^{-5} gL^{-1}) and a double salt concentration to obtain an isotonic medium. The extracts were centrifuged (3000 rev min^{-1}) for 30 min. The supernatant was collected for the bioassay of ACh on the rectus abdominis of the frog. The procedure given by the Staff of the

Table 1. Action of achronic arecoline administration on mouse motility and on the ACh levels in two sections of the central nervous system.

		Controls	Arecoline 4·5 mg kg^{-1}	Arecoline 9·5 mg kg^{-1}	Arecoline 28 mg kg^{-1}	Arecoline 60 mg kg^{-1}
		Number of squares traversed in five min				
× ± S.E.		57·20 ± 3·82	52·40 ± 2·81	50·10 ± 2·61	44·65 ± 1·95	40·37 ± 5·55
n		20	20	20	20	20
P		—	40%	60%	1–0·1%	1–2%
		Acetylcholine levels (μg g^{-1} fresh tissue)				
× ± S.E.		2·51 ± 0·31	2·35 ± 0·15	3·04 ± 0·16	3·39 ± 0·18	2·61 ± 0·08
n	Cortex	5	7	5	7	7
P		—	60%	20%	20%	80%
× ± S.E.		3·45 ± 0·33	2·95 ± 0·19	3·75 ± 0·46	5·38 ± 0·28	5·35 ± 0·28
n	Subcortex	5	7	5	7	7
P		—	20%	60%	1–0·1%	1–0·1%

n = number of animals; P = probability of a casual result for the differences to the controls.

Department of Pharmacology, University of Edinburgh (1969) was followed.

Results and discussion

Based on the mean fluid consumption and on the mean body weight during the experiment, the doses of arecoline administered to the 4 experimental groups were respectively 4·5; 9·5; 28·5; and 60 mg kg^{-1} day^{-1}. The three lower doses in our experiments are in the range of the doses (6, 12 and 20 mg kg^{-1} day^{-1}) used to study memory retention in a chronic arecoline administration (Flood et al 1984). The dose of 60 mg kg^{-1} day^{-1} is similar to the dose (50 mg kg^{-1} i.p.) used by Haubrich & Watson (1972). 60 mg kg^{-1} is certainly a high dose of arecoline considering that the LD_{50} for an acute administration in the mouse is between 84 and 68 mg kg^{-1} i.p. (Molinengo & Orsetti 1986). But it must be noted that in our experiments the 60 mg kg^{-1} were consumed over 24 h and there is evidence that the duration of effect arecoline is short (15–20 min) (Pradham & Dutta 1970), suggesting a rapid drug elimination.

In Table 1, the means and the standard errors of the number of squares traversed by the mice in the 5 min of experiment are given together with the levels of ACh found in the cortex and subcortex of mice which received arecoline for 20 days. Student's *t*-test was used to test the significance of the differences between controls and treated mice. Arecoline caused a reduction of the number of squares traversed in the open field only at the higher doses (28·5 and 60 mg kg^{-1} day^{-1}). At lower doses no statistically significant modification of behaviour was observed. Pradham & Dutta (1970) reported a depression of the rat spontaneous activity after acute administration of a much lower dose (2 mg kg^{-1}). Tolerance to the depressive effects of arecoline might explain this discrepancy, although in our experiments the animals drank arecoline solution and the dose assumed was distributed along 24 h.

Our results indicate that at low doses of arecoline (4·5 and 9·5 mg kg^{-1} day^{-1}) there is no modification of the levels of ACh in the cortex and subcortex. Only with higher doses (28·5 and 60 mg kg^{-1} day^{-1}), which also reduced mouse motility, was an increase of the ACh levels in the subcortex observed. These observations suggest that with a chronic arecoline administration, the depression of mouse motility is concomitant to an increase in the ACh levels in subcortical structures. These effects, being observed at rather high doses of arecoline, may be considered an aspect of chronic arecoline toxicity.

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